Observations on the Trypanosome of the Little Owl (Athene noctua), with Remarks on the other Protozoan Blood-parasites occurring in this Bird.[1]

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With Plates 20 and 21.

The observations to be recorded in this and in a subsequent paper are the result of a study of the protozoan blood-parasites of the little owl, which was undertaken by us at Rovigno, Istria, the actual place where Schaudinn's celebrated investigation (**16**) on the same parasites was carried out. A re-investigation of these parasites has long been considered urgent, and stress has been laid by many people upon the importance of the work being done at Rovigno itself. The different views which have been taken with regard to the correctness or otherwise of Schaudinn's account are now well known, and it is scarcely necessary to recapitulate them here, more especially since they have been recently discussed by one of us in the first memoir of this series (**18**).

We were together at Rovigno for nearly three months, from about the middle of January, 1909. Unfortunately, we went much too early in the year. This was chiefly due to the fact that one of us (E. A. M.) was obliged to be in London from May onwards in order to deliver the University course of lectures, which had been previously arranged for

[1] "Studies on Avian Hæmoprotozoa," No. II. For No. I see 'Quart. Journ. Micros. Sci.,' vol. 55, p. 641.

that period. The other of us (H. M. W.) stayed on at Rovigno, continuing the work alone, until the beginning of July, when, after a stay of six months, he was also obliged to return on account of other work. There can be no doubt that if our visit to Rovigno could have been arranged for the six summer months, say from April to September, it would have been much more successful than it was. Still, we are able to bring forward certain definite observations, which may contribute towards the settlement of this difficult and much-debated question.

We take this opportunity of expressing our very grateful acknowledgments and thanks to the various people through whose courtesy and kindness we were enabled to undertake and prosecute this research; we were especially indebted to the late Dr. Hermes, the Director of the Zoological Station at Rovigno, for placing laboratory accommodation at our disposal, and to Dr. Krumbach, in charge of the Station, for his great assistance in endeavouring to obtain the owls.

The Birds and their Parasites.

At the outset we experienced a sad disillusionment in connection with the supply of owls. Although little owls were not scarce in the district, they were extremely difficult to procure. In the course of a month, in spite of all our efforts, we could only obtain five birds locally, which were brought in mostly in a wounded or dying condition after being shot. We tried several times to find haunts or nesting-places of the owls, but without any success. These difficulties in regard to the local birds did not affect us so much, however, as we feared at first would be the case, because (somewhat to our surprise), none of the above five birds showed signs of any hæmoprotozoan infection at all after thorough examination. After we had been at Rovigno a month we managed to obtain some owls from Vienna which were infected; and thence-forward we relied altogether upon dealers in Vienna and also in Breslau for our supply of birds. The only other local owl

examined, in fact, was a young fledgeling, brought into the laboratory about the middle of June. This little bird, which went by the name of "Piepsch," also had no parasites in its blood; it, too, was most probably quite free, but it became such a favourite that its sacrifice to science was not permitted. From our experiences with Rovigno owls we consider it quite likely that the birds in this neighbourhood were not infected with the protozoan blood-parasites specific to them; on the other hand, we never had more than two consecutive birds sent from Vienna or Breslau which were quite free from parasites.[1]

A great difficulty with which we had to contend was that the climate of Rovigno did not suit the inland owls, confined as they were in cages. As regularly as the periods occurred when the moisture-laden Sirocco prevailed, equally regularly did one or more of our precious owls die suddenly; and this danger of losing all at once an important bird continued to be a source of anxiety the whole time we were there. As a matter of fact the investigation would probably have been rendered much easier had we gone to Vienna to carry it out. There could not have been the slightest objection to choosing this district in preference to Rovigno, for in the course of our stay we gathered from Giovanni, one of the sailors attached to the laboratory, who was there in Schaudinn's time, that most of the owls which Schaudinn himself used came from Vienna.

Altogether we had eighteen owls sent from Vienna or Breslau, the birds being more readily obtained as the spring advanced. The accompanying table has been drawn up to show at a glance the number of birds infected, and the different parasites present in each case. (Tryp. = Trypanosoma, Halt. = Halteridium, Lz. = Leucocytozoon, and Prot. = Proteosoma.)

[1] Since our return home we have had a batch of four young owls sent from Geneva, all of which have proved entirely negative. This may be also an uninfected district.

Owls.			Parasites.			
No.	From	Date.	Tryp.	Halt.	Lz.	Prot.
6	Vienna	Jan. 26	—	—	—	—
7	„	Feb. 21	?	+	—	+
8	„	„ „	+	+	+	+
9	„	„ „	—	—	—	+
10	Breslau	March 17	—	—	—	—
11	„	„ „	—	+	+	+
12	„	„ „	—	—	—	—
13	Vienna	Apr. 16	+	+	+	+
14	„	„ „	+	+	+	—
15	„	„ 27	—	—	—	—
16	Breslau	May 8	—	—	—	—
17	Vienna	„ 23	—	—	+	+
18	„	„ „	—	—	+	—
19	„	„ 29	+	+	+	+
20	„	June 5	—	—	+	—
21	„	„ „	—	—	—	—
22	„	„ 12	—	—	+	—
23	„	„ „	+	+	+	+
Total, 18			5 (or 6?)	7	10	8

Analysis and General Remarks.

Twelve out of the eighteen birds were infected with one or more parasites, the remaining six being, so far as could be ascertained, quite negative. Of the different forms of parasite the Leucocytozoon occurred most frequently, being present in ten instances. Hence, more than 55 per cent. of the owls we obtained were infected with this parasite—a high ratio. Proteosoma was present in eight birds, i. e. in more than a third. Halteridium occurred in seven owls, and trypanosomes in either five or six. It will be noticed that no trypanosomes were found in any of the birds from Breslau, but only in those from the neighbourhood of Vienna.

With regard to the number of owls in which trypanosomes were present, we think it quite possible that owl 7, marked in the table with a query, may also have been infected, although trypanosomes were not actually found. Halteridia were also very scanty in owl 7. On the other hand, in owl 8 Halteridia, as well as most of the other parasites, were plentiful, and on

the death of this bird trypanosomes were readily found in its bone-marrow. When owl 7 died a few weeks later its bone-marrow was examined carefully, but no trypanosomes were seen and other parasites were also very scarce ; consequently, only very few preparations were made from it since we regarded it as negative in respect to trypanosomes, and we had a number of good preparations from owl 8. The one smear of bone-marrow made from owl 7 had, unfortunately, very little on it, and was not much use ; hence, and in view of our experience with owl 19, presently to be described, we cannot feel at all sure that trypanosomes were not present in owl 7, perhaps scanty in number.

Owl 11 had a good infection with Leucocytozoon, and Halteridium was also present ; the latter form was excessively rare, however, only a few flagellating gametocytes being seen in the course of many living examinations, and the parasites being extremely difficult to find in permanent preparations. No trypanosomes were found in the bone-marrow of this bird. On the other hand, in both owls 13 and 14, the next birds obtained which had a good (or fairly good) infection with Halteridium, and in which Leucocytozoon also occurred, trypanosomes were found after death without much difficulty, as in the case of owl 8. Owl 19 also had a good infection with Halteridium, and when this bird died later on it was expected that trypanosomes would be found in it, as in the others; but in spite of very careful searching of fresh preparations for a period of two hours no indications whatever of trypanosomes were seen ; a colleague, Dr. Reichenow, who was then working at Rovigno, also kindly examined a preparation without finding anything. Hence it was thought that in this case the Halteridium and the trypanosome of the owl had been definitely separated from one another, and that this was a case of infection with the former parasite only. However, good preparations from the organs of this bird were made for the sake of the Halteridium and Leucocytozoon, and in the systematic search of these undertaken since returning home, three small trypanosomes have been found in four

smears of the bone-marrow! The parasites were evidently extremely scanty; one of us, notwithstanding a fair amount of experience with Avian Trypanosomes, has never had another bird in which these parasites were so rare in the bone-marrow.

The incident of owl 19 emphasises the point that it is very difficult to be certain, from a living examination alone, that trypanosomes are absent from the bone-marrow. Small forms especially may be quite hidden by clumps of cells. The spreading out of a thin smear for a permanent preparation helps to set free and disclose the parasites.

The constant association of trypanosomes with one or more of the intra-cellular parasites, especially the Halteridium and Leucocytozoon, is very striking. In no case did we find trypanosomes in an owl which had not these two other forms also. Nevertheless, suggestive as this fact may be, we have been unable to obtain any evidence which would point to anything being concerned beyond coincident occurrence of these different types of parasite. Moreover, taking the other forms first, the coincidence is somewhat less marked. Thus, while five (possibly six) of the seven birds infected with Halteridium also had trypanosomes, in one certainly (No. 11) trypanosomes were not present; it is true that here the Halteridia were excessively scanty. Again, with regard to Leucocytozoon, this intra-cellular parasite occurred in five birds in which no trypanosomes could be found. In the case of three of these, Nos. 11, 17, and 22, in which the Leucocytozoon was either fairly frequent or else plentiful, good smears from the bone-marrow and also from the other organs (in the case of No. 22) have been well searched with negative result.

As will be gathered from the table, Proteosoma occurred indiscriminately, either alone or together with one or more of the other parasites; while in owl 14, which had the other three forms, Proteosoma was absent. An interesting point of difference may be mentioned between Proteosoma on the one hand and Halteridium and Leucocytozoon on the

other, the possible significance of which will be discussed further. In the early owls Proteosoma occurs chiefly in the form of schizonts; up to No. 11 inclusive, scarcely any gametocytes were found. In the later owls, on the other hand, the parasites were mostly in the gametocyte phase, schizonts being absent or rare. Halteridium and Leucocytozoon, on the contrary, occurred in the gametocyte phase in the earliest owls in which they were found—Nos. 7 and 8; many individuals were apparently ripe, since they "flagellated," or became rounded and free, according as they were of male or female sex, in the fresh coverslip preparations. Indeed, so far as these two intra-cellular parasites are concerned, we may take this opportunity of saying that, as regards endogenous multiplication or schizogony, we have been quite unable up to the present to obtain any evidence of such a process, and this in spite of much searching, since our return home, of what we considered very promising material.

Owl 23, for instance, had the strongest Halteridial infection that either of us has ever observed; nearly every red blood-corpuscle was infected with three or four—sometimes more—of these parasites, the majority of them being small forms. Whence this host of parasites originated remains a mystery; neither smears nor sections of lung and spleen have as yet shown anything different from the condition found in the general circulation. And the same result has attended the searching of preparations of the organs of earlier birds, where the Halteridia were not so abundant in the blood. The point remains for future investigation.

Habitat.

Turning our attention now more particularly to the trypanosomes, it may be remarked in the first place that, so far as our observations have gone, the trypanosome of the little owl shows a general correspondence with that occurring in the chaffinch and redpoll, described in the first memoir. This is

the case both as regards the habitat of the trypanosomes and their behaviour in the bird at different seasons, and also in regard to the chief morphological types found.

In the winter and spring months the bone-marrow is the chief seat of the parasites; indeed, for all that we could learn to the contrary, they may be restricted to this situation. The blood of owls 8, 13 and 14 was examined living on many occasions, both in the day-time and at night, and numerous stained preparations of the peripheral blood and the internal organs (lungs, spleen, etc.) have been searched, without in any case coming upon a trypanosome.

Nevertheless, from the experiences of one of us in reference to this point, in working on other birds, we should not like to say certainly that the parasites do not occur at all in the general circulation at this period.[1] Most unfortunately the indispensable test, that, namely, of taking cultures from the blood, was quite a failure in its application in this case. Several culture-tubes were inoculated from the peripheral blood of the above owls, which remained uncontaminated, or practically so, by bacteria, but they were also sterile as regards trypanosomes. The disappointment was, however, that in tubes inoculated from the bone-marrow of these birds, in which trypanosomes were known to be present, no cultural forms developed. In no instance, in fact, did we succeed in obtaining a culture of the owl-trypanosomes, a very different result from the usual experiences with the culture-method. As Rosenbusch (**15**) has obtained cultures of the parasites—i. e., as will be shown later, of the trypanosomes—of the little owl, we can only surmise that the fault in our case lay with the tubes used.[2]

[1] Mayer, however, has noted (**12**) the occurrence of the parasites (in the large, massive form, c.f. below) in the general circulation in the early part of the year (January) in the case of the trypanosome of Syrnium aluco.

[2] We had no facilities for making culture-tubes at Rovigno, and had to rely upon some taken with us; many of them were rather old by the time they were required; others had to be boiled up and fresh salt-

On the other hand, in the summer, for instance in owl 23, the trypanosomes certainly occurred in the general circulation. They were seen for the first time in blood taken from the living bird on June 15th–16th, in the night-time, after being five months at Rovigno! This fact is mentioned just by way of illustrating the difficulties attending the path of the would-be researcher on these Avian parasites. The trypanosomes were found also in subsequent examination of the bird; and in permanent preparations made they are not infrequent, averaging four to six on a good-sized film. Even at this time, however, the parasites are somewhat more numerous in the bone-marrow, as we have learnt from our preparations made after the death of this bird (which took place on June 29th), while they appear to be extremely scanty in the smears made from the internal organs (lung, spleen, etc.).

Living Observations on the Parasites.

With a view of ascertaining whether we could find anything which pointed to the actual passage or transformation of the trypanosomes into Halteridia or Leucocytozoa, or vice-versâ, careful living observations were made on several occasions, usually in the night-time.

A. Trypanosomes.—The following notes were made at the time of the examination of the bone-marrow of owl 8 after its death (February 26th, 1909), in which, of all our infected birds, trypanosomes were most numerous to this situation. The parasites observed were of three distinct kinds: (1) Slender, active forms, of medium size, with a finely drawn-out aflagellar extremity. In two instances such forms were seen attached by this end to a cell of leucocytic character; the trypanosomes were lashing about in every

citrate added. Our tubes were, therefore, not very satisfactory; but we were not prepared for the entire failure of the trypanosomes to appear in them, considering the readiness of these Avian parasites, as a rule, to develop in cultures.

direction, apparently unable to free themselves, and were dragging the cell here and there. (2) Large, stout, sluggish forms, with a very long, tapering aflagellar end, and a much pleated undulating membrane; the free flagellum was short. These individuals travelled very slowly, scarcely moving from one place; they often seemed to get wedged in among clumps of cells. The membrane, however, was in a continual state of rippling, and an interesting point was noticed in connection with this, namely the reversal of the movement. The waves ran usually from the region of the kinetonucleus towards the flagellum, and the movement in this sense was continued for the longer period; but it was frequently seen to change, when the waves ran from the flagellar end towards the kinetonucleus, for a short time. All of a sudden, and at irregular intervals, the movement would change again and be once more in the original direction. (3) The third type of parasite seen was quite a small form, not much longer than a blood-corpuscle; these individuals were somewhat stumpy in appearance, with the aflagellar end abruptly pointed. These forms were not noticed at first; they were probably overlooked through being hidden by or among clumps of cells, out of which they managed later to worm their way. They had a wriggling movement, but did not seem to travel much. These small trypanosomes appeared to have a predilection for attaching themselves by the tip of the flagellum to the surface of the glass, either to the slide or the cover-slip.

On examining a preparation the following morning (some ten hours later), one of the small forms was observed slowly writhing about, quite free from any corpuscles, but with the tip of its flagellum firmly attached to the slide by a distinct dot or granule. The trypanosome, by its movements, pushed and pulled its body from and to the spot where it was attached, without, however, becoming free. The membrane undulated slowly; its flagellar border appeared distinctly to be wound spirally round the body of the parasite. The body itself seemed somewhat stiff, and only changed its curves slightly and slowly. Another trypanosome, probably belonging

to the first type mentioned, was also noticed at this time; it was recognised by its movement, but as it was more or less surrounded by a clump of corpuscles, its characters, with the exception of its approximate size, could not be made out. Looked at again later (about noon) the small trypanosome was found to have detached itself, and was wriggling feebly, loose, and waving its flagellum about slowly; it appeared moribund. At 2 p.m. it was nearly dead, only the tip of its flagellum now moving feebly.

The same three types of individual were also found in the bone-marrow of later owls (Nos. 13 and 14); and here again, in the latter bird, certain trypanosomes were seen to be distinctly attached. A medium-sized, slender form (belonging to the first category) was attached to a corpuscle by its flagellum, by means of which it was waving the corpuscle about vigorously. Further, a small trypanosome (type 3) was seen unmistakeably attached by its flagellum to a leucocytic cell; and another small individual also appeared attached, but this could not be ascertained with certainty owing to the parasite being partially hidden. These observations were also made in the evening, about 8 p.m.; and the above two individuals were watched at intervals until midnight without any change being noticed. Next morning the slender form could not be found again; while the small individual was quite motionless and seemed to be dead.

From the above notes the following definite and rather interesting points may be emphasised. The slender, medium-sized parasites, and also the small, stumpy forms, may be found attached to a cell, either by the flagellum or by the aflagellar end. On the other hand, no individual belonging to the large, sluggish type (of the second category) was ever observed attached.

In owl 23 quite a different state of affairs was met with from that obtaining in the earlier owls. The trypanosomes in this bird were in what we propose to refer to as the "summer condition"—both as regards the habitat of the parasites and the type of form found. This condition is to be

recognised, we think, as constituting a perfectly definite phase in the life-history of these Avian trypanosomes. As above mentioned, the parasites were observed living in the peripheral blood on two occasions, in the night-time; only very few trypanosomes were noticed altogether in several cover-slip preparations. They belonged to one type, and were fairly small and stumpy, spindle-like or rather broad; as will be seen later, these forms are not quite comparable to those of the third category above described. All the trypanosomes observed living in this owl, whether in the blood or (later) in the bone-marrow, were free; none showed any inclination to become attached to any cell.

To sum up: In spite of many and long-continued observations we never succeeded in seeing any form of the trypanosome of the little owl either penetrate into, or become completely attached to a cell, and lose concurrently its locomotor organs; and this notwithstanding that both Halteridia and Leucocytozoa were present in all cases in the same birds. The only manner in which we observed the trypanosomes to be associated with cell-elements was that of their attachment by one extremity—which might be, apparently, either end indifferently; and we never saw this attachment develop into any closer connection (cf. however, below, p. 165).

B. Leucocytozoon.—We do not intend in this paper to discuss the minute structure of these parasites, but only to note certain features observed, and more especially those bearing upon the question of the actual connection of these intra-cellular parasites with the trypanosomes. Owl 11 had a good infection of Leucocytozoon and was the most suitable of the earlier birds for studying this form. The parasites were all in the gametocyte-phase, most of them being nearly full-grown in size. In nearly all the host-cell had acquired the characteristic spindle-like appearance, with the cytoplasm drawn out in two long, tapering processes. The degree of plumpness of the parasites varied slightly; some were more slender, others rather stouter; but apart from

this, there was strikingly little difference to be noticed among the numerous parasites observed. We certainly never saw anything resembling the curious appearances and behaviour of the parasites described by Dutton, Todd and Tobey (**3**) in another species of Leucocytozoon; and in this respect our observations concur with those of Wenyon (**17**).

Many individuals were watched very carefully for signs of movement, but in no instance did we see any active movement or change of form, either of the ovoid, more internal (endoplasmic) part of the parasite or of the tapering extremities of the spindle. The latter, probably consisting at any rate in the narrower portions only of the cytoplasm of the host-cell, were sometimes seen to bend slightly to and fro, quite passively, this motion being caused, doubtless, by little currents in the blood-plasma in the immediate neighbourhood. We never observed any amœboid movement or anything comparable to the waves of constriction described by Wenyon in the case of L. neavei; neither did Mathis and Léger (**8** and **10**) in their form from the fowl. Of course, in referring to this absence of movement in the parasites, we are not taking into account the preliminary efforts of ripe gametocytes either to rupture the host-cell or to form microgametes. It is possible that the observations made by Wenyon may refer to such ripe gametocytes which were endeavouring to free themselves from the leucocytes; though it is true we always found this process to occur very rapidly in L. ziemanni. The reason why we have laid stress on the entire absence of active movement in the ordinary spindle-shaped individuals—which was always the case in every bird in which we studied them—is to emphasise the fact that we never saw any indication whatever of the development of locomotor organellæ, or of any active trypaniform phase. Further, we have never once found, either in living preparations or in the permanent smears, any young or intermediate-sized individuals or forms other than the ripe gametocytes, free in the blood-plasma, however numerous the parasites were.

An interesting feature which may be pointed out was the variation we noticed, both in the number of gametocytes present in the drops of blood examined and in the apparent ripeness at different times. Mathis and Léger (9) have remarked on the occurrence of what they describe as a periodicity of the gametocytes in the circulating blood, which they noticed on two occasions in Leucocytozoon caulleryi, of fowls in Tonkin. The parasites would be observed for a certain number of days (four to seven) in varying frequency, and then would apparently disappear from the blood and not be seen again, often for some weeks. In the cases instanced there appeared to be no regularity either about the length of time during which the parasites were not observed, or about the period, usually a few days, when they were present.

We never observed in any of our owls infected with Leucocytozoon a complete disappearance of this parasite from the blood. The following notes illustrate the variation to which we have just alluded in owl No. 11.

March 18th, morning after arrival: Leucocytozoon present; parasites frequent.

March 19th–20th, midnight: Parasites distinctly more numerous, approaching abundance. Many rounded-off forms (i.e. ripe females, which had ruptured the host-cell) seen. Probably ripe male individuals were also present, though no flagellating forms were actually seen.

March 23rd, morning: At first no signs of Leucocytozoon, but after some searching the parasites were found. They were infrequent to scanty, only five or six individuals, one of which was rounded-off, being noticed in four cover-slip preparations.

March 23rd–24th, midnight: Parasites numerous. In a cover-slip preparation of pure blood only spindle-shaped, intra-cellular forms were seen; but in a preparation to which a drop of salt-citrate solution had been added, many liberated females and some flagellating male gametocytes were found.

March 27th–28th, midnight: Leucocytozoon not at all frequent, rather scanty in fact, requiring considerable searching. Parasites distinctly in batches. In one drop none were seen after much searching, while in another four or five intra-cellular forms were come across one after another fairly quickly. In a drop diluted with salt-citrate solution two ripe females and also two flagellating males were found.

March 30th, morning: Leucocytozoon numerous. Many rounded-off females observed, but only one flagellating male.

April 3rd, afternoon: Owl 11 died. Leucocytozoon numerous, both in blood and internal organs. No rounded-off or flagellating individuals were observed, however, whether in preparations to which salt-citrate had been added or in those to which it had not been.

In several of our other owls in which Leucocytozoon was present (e.g. Nos. 14, 17 and 19), the parasites were infrequent or scarce whenever the birds were examined (this being the case even in the internal organs after death), and consequently no such variation in number was observed. In owl 22, however, which had a very good infection with Leucocytozoon, the above phenomenon was again noticed.

June 13th, morning after arrival: Leucocytozoon numerous; many flagellating as well as rounded-off individuals seen.

June 21st–22nd, midnight: Parasites not nearly so numerous, only fairly frequent, and no flagellating or rounded-off individuals were observed.

June 22nd, afternoon: Owl 22 died. Both in the heart-blood and in the bone-marrow Leucocytozoon was fairly numerous, distinctly more frequent than in the peripheral blood the night before, but again no ripe forms were seen—either rounded-off or flagellating.

Of our birds infected with Leucocytozoon, the one which we had under observation for the longest period was No. 14, which was first examined on April 20th and lived till May

20th—just a month. Leucocytozoon was very scarce in this bird. It was not found in the living examination on April 20th; but on April 23rd a single individual was noticed in two cover-slip preparations. Examined again on May 9th (morning) and also on May 12th–13th (midnight) no Leucocytozoa could be seen in the living drops. But a very few individuals have since been found on searching permanent smears made on these occasions. Hence the parasites were present in the general circulation, though so scanty that their presence could not be demonstrated in the routine examination of several living drops on both occasions. Further, in the smears made from the heart-blood of the dead bird on May 20th Leucocytozoon is also present, though very scanty.

From the above observations we think, therefore, it is practically certain that in none of our owls infected with Leucocytozoon was this parasite at any time really absent from the general circulation.

The variation in number and ripeness of the gametocytes, and their occurrence at times in distinct batches, is most probably the result of some antecedent process of schizogony, by which the sexual forms have originated. A schizogonic mode of multiplication has been briefly described by Fantham (**4**) in L. lovati of the grouse; and a similar phase occurs in all likelihood in other Leucocytozoa. At a particular moment, we may suppose, a number of young (potential) gametocytes are liberated by the breakdown of the host-cell in some internal organ in which they have been developed. They penetrate, probably as soon as possible, into the new leucocytic host-cells in which they will grow and mature, and so pass into the blood-current, in which they are passively borne along. As it is quite probable that different host-cells (or groups of host-cells) containing the products of schizogony become ruptured at different times, we should have, in that case, clumps or batches of gametocytes of slightly different age and ripeness in different small quantities or volumes of blood.

The only other explanation of our observations—on owl 11, for instance—would be that the great majority of the numerous gametocytes observed on March 19th–20th had died off by the 23rd, and that those seen again on March 23rd–24th were an entirely fresh lot, which had been very rapidly developed as the result of a quite recent schizogony. In the first place it is most unlikely that the gametocytes would die off in such numbers as soon as, or even before, they were quite ripe, especially at the beginning of the season—such a course would be very expensive and most unusual for a parasite. Secondly, if schizogony had been going on recently to produce the numerous forms seen in the blood March 23rd–24th, and again, subsequently, to give rise to those present on March 30th, we should certainly have expected a proportion of these forms to be small to intermediate in size, which is not the case; and, moreover, we ought undoubtedly to have found some indications of the actual schizogonic process in our permanent preparations of the internal organs made on April 3rd, when the bird died. As a matter of fact in all our infected owls the schizogonic process seemed to be over and done with; altogether we have only come across extremely few really small (young) gametocytes. Hence, the first explanation we have offered appears by far the most reasonable.

It is interesting now to compare with our experiences those of Mathis and Léger in the case of the species parasitic in Tonkin fowls already alluded to. It is important to note that the periodicity described (occurrence for some days, alternating with apparently complete disappearance for a period of two or three weeks or more) was observed only in L. caulleryi; in L. sabrazesi from the same bird, which was made a distinct species on account of the gametocytes always having the spindle-like shape, this disappearance was not found. In a previous memoir (18) it has been remarked by one of us that those two species are most probably only different phases of one and the same parasite. The L. sabrazesi phase probably corresponds in a general way

to the condition in which we found L. ziemanni in some of our owls. The L. caulleryi phase, on the other hand, appears to represent an older condition of the parasites—probably a much older infection—in which the gametocytes are quite mature, it may be, over-ripe. In such a case one may suppose that many of those which do not succeed in passing into the alternate host at length die off. It is not unlikely, we suggest tentatively, that others are able to undergo some parthenogenetic development and give rise later to a fresh succession of gametocytes in the blood. In this manner the reappearance of the parasites after an interval could be readily explained.

We may conclude our remarks on Leucocytozoon ziemanni by giving extracts from our notes relating to one or two interesting observations on the gametes, which we were fortunate enough to find in living preparations. The male gametes were first detected by the movements of the corpuscles which they caused. They appeared as very slender, spirochæte-like bodies, exceedingly active, performing twisting movements and travelling at a fair pace; they were rather longer than a red corpuscle and capable of jerking the corpuscles about. Three or four of these delicate elements were seen in an area rather larger than a field of the microscope. In another field an active male gamete was found and also a rounded-off female individual; the latter was quite spherical and had ruptured its host-cell, the remains of which, together with the nucleus, were still attached to one side of the parasite. The male gamete was at first some little distance from the female element; it travelled fairly fast, and in keeping it in view the female individual was sometimes quite out of the field (of the oil-immersion lens), and then would be brought in again, i.e. the microgamete was sometimes nearer to, at other times farther from, the female. Once the male was seen to travel in a straight line rapidly towards the female till it nearly reached it; but then it turned off to one side again. After moving about a little longer, however, the microgamete at

length approached the female gamete, and after some gyrations, which caused the latter to be jerked about as well as the corpuscles in the vicinity, it was seen to be definitely attached to the female by one extremity. The male element continued to lash its body and jerk the female about, but after a time these movements became feebler and the male seemed to be contracting. At this moment the female was suddenly violently jerked, and after that no further movements were seen on the part of the parasite. The female individual was now examined very carefully but no signs of any little body attached to it could be made out; the male gamete seemed to have been absorbed. At this period no definite nucleus could be distinguished in the female; one part of the body was clearer and free from pronounced granules, but no sharply contoured nucleus could be made out. After watching the parasite for some time it was noticed that a red corpuscle, flowing slowly past it, was deflected slightly from its course, and it was seen that there was then a small body attached to the parasite at this point; this little body was spherical, of a definite contour, and contained a few granules; it appeared like a minute cell or nucleus. Shortly after this minute body had been found and when our attention was again turned on the parasite proper, it was observed that its nucleus was now quite distinct. It could be seen as a slightly oval clear space, with sharp and definite contour, and had near one end a dull spot, quite different in appearance from the much darker grains in the cytoplasm. The diameter of the nucleus was about double that of the little body attached to the female gamete. The parasite was watched for some time longer, but the only change observed was that the attached body seemed to become rather contracted and shrivelled, as if degenerating. No indications of any other change or development in the parasite itself could be noticed. When seen again in the morning (of the same day) the Leucocytozoon had become hyaline in appearance and seemed to be dead; there was no sign of the little attached body.

This was the only occasion on which we were able to see the fertilisation of a female gamete of Leucocytozoon, but several times we observed the male gametocyte in the act of flagellating, i.e. of developing the microgametes. This process occurred more readily in coverslip preparations to which a drop of salt-citrate solution had been added than in those put up of pure blood alone. When the microgametocyte ruptured its host-cell its body protoplasm was usually more or less segmented or divided up into two or three lobes or portions from which the male elements were given off, just as was described and figured by Schaudinn (loc. cit.). The number of microgametes formed appears to be variable. Schaudinn gives the number as eight; on the other hand, Laveran (7) figures four as arising, also in this species of Leucocytozoon ("Hæmamœba" ziemanni). In one case we saw three quite distinctly, and there may have been a fourth, but we could not be certain; in another instance, where the body of the parasite (freed from the host-cell) had been constricted into two masses, only one male element was seen to be formed. It is not improbable that in the citrated drop some gametocytes may be stimulated into attempting to develop microgametes before they are really quite mature enough to do so in a completely normal manner. Thus in the last instance given, the solitary microgamete, at first flagellum-like and active, appeared unable to liberate itself from the protoplasmic mass, and after five or six minutes its wrigglings became less active and more spasmodic, and finally it became much contracted and pear-shaped and ceased to move. In other cases, again, the microgametocyte did not succeed in rupturing the enclosing envelope of the host-cell, and the microgametes were developed inside the skin or capsule, as it were, of the leucocyte, from which they were unable to get free. In one instance several male elements (there may have been as many as eight) were seen thus imprisoned; they were in two bunches, directed towards the spindle-like ends of the host-cell, and were lashing themselves about vigorously in the

endeavour to become free. This movement went on for about three quarters of an hour, but with no success.

The free microgametes themselves were, as already mentioned, very slender thread-like bodies; they were, if anything, rather longer than those of Halteridium. The gametes, living, were observed very closely, but no signs of any undulating membrane could be made out; nor could any more active, whip-like part of the body, corresponding to a free flagellum, be distinguished. Unfortunately, we have not been able to find any microgametes in our permanent preparations; Wenyon, however, has figured (**17**) the male elements of L. neavei, from a stained preparation, and these also appear simply as threads. It is most likely, we think, that the minute structure of the microgametes of Leucocytozoon is very similar to that of the corresponding elements in Halteridium; in the latter parasite, the male gamete, as has been described by one of us (loc. cit.), consists of a delicate cytoplasmic thread, containing two or three chromatic masses of varying size and having a distinct centrosomic granule at one extremity (cf. also below).

c. Halteridium.—Three of our owls had a good Halteridial infection, Nos. 13, 19, and 23; the parasites were plentiful in the first, abundant in No. 19, and simply swarming in No. 23. It may be remarked here that the terms "numerous," "abundant," and so on, as we have used them, do not mean the same thing, as regards the actual number of the parasites present in the case of Halteridium and Leucocytozoon respectively; this will be understood when the different habitat of the two forms and the relative proportion of red blood-cells to small mononuclear leucocytes is borne in mind. For instance, we may consider Leucocytozoon to be numerous when two or three individuals on an average can be seen in a single field of a fresh cover-slip preparation, working with a dry lens (obj. D or 4 mm. apochromatic); but we should not regard Halteridium as abundant in an infected bird unless on an average at least one or two individuals occurred in a single field of a stained smear,

using an oil-immersion lens. Such an infection was present in owl 19. And in owl 23 nearly every red blood-corpuscle is infected; very few uninfected red cells can be found in the permanent preparations; there are nearly always two or three parasites in a single host-cell, and frequently their number is four to six, when they are mostly small or quite minute forms.

No pronounced variation in the number of the Halteridial parasites present on different occasions of examination was observed, contrary to what was so markedly the case in Leucocytozoon. It was observed several times, however, that there was distinct, often considerable variation in the number of individuals which were ripe enough to flagellate or become rounded off. Thus, at some examinations, by the time a drop of pure blood could be mounted and put under the microscope numerous male gametocytes would be seen actively liberating free microgametes, rounded-off female forms also of course being present; at other times scarcely any such, or none at all, would be found. Another noticeable point of difference from Leucocytozoon was that not only adult or nearly adult individuals, but also young or small forms and forms of intermediate size, were nearly always present at the same time in the blood.[1]

On the whole, comparing the results of our observations on Halteridium and Leucocytozoon, we think the following conclusions are suggested. The schizogonic process in the former parasite must be on a considerably larger scale than it is in the latter, even if we suppose that there is a stronger original infection.[2] Further, it is probable that the schizogony in Halteridium may be of longer duration, i. e. that it may continue to go on for a longer period than is the case in the Leucocytozoon; this seems to us to be indi-

[1] This agrees with the condition which was found in the case of H. fringillæ, in the chaffinch.

[2] If schizogony in the parasite of the little owl is similar to the process described by Aragao (1) in the Halteridium of the pigeon, it is indeed on a lavish scale.

cated by the fact that in our early owls as well as in our later ones quite small forms, which cannot have been long set free from the parent schizont, occur as well as others of medium size in the red blood-cells; also because the later the bird, the greater the number, as a rule, of Halteridia present (owls 8, 13, 19 and 23 form a regular series in this respect).

In the birds in which Halteridium was fairly numerous or abundant (Nos. 13, 19 and 23) parasites were seen in the living cover-slip preparations which were quite free from a blood-corpuscle, although they were not rounded-off or flagellating individuals. Only a few of these free Halteridia were noticed in owl 13, they were less scanty in No. 19, while in No. 23 they were quite common. In the last case these free forms varied considerably in size, from small individuals up to forms of intermediate size or larger. These free forms appeared, so far as could be seen, perfectly similar to those in the corpuscles. It is important to note that they were quite motionless and were not observed to undergo any change. Particular individuals, fairly large ones, were watched for two or three hours at intervals, and at the end of that period had not altered at all. Not the least indication was seen of the development of any of these free Halteridia into a trypaniform condition. In the living blood from owl 23, examined at night, one or two trypanosomes were found; these were distinctly larger than the free Halteridia. In spite of much searching, no parasites were seen to become actually liberated from the corpuscles. It is certain, however, that most, if not all, of these free individuals had been parasitic in a red cell, for the great majority contain pigment-grains.

The same state of affairs observed in the living preparations from these three owls is found in the permanent smears made from them respectively. Here and there in the preparations from owl 23, where, as already mentioned, several parasites often occur in a single corpuscle, three or four free individuals are found grouped around or else close to the isolated nucleus of a red cell; in such cases one may assume

that the cell has been disorganised artificially in making the film. Most of the free forms are more or less uniformly dispersed, however, and have no broken-down remains of a red cell in their vicinity. This fact, and, of course, the occurrence of these forms in the fresh preparations, makes it certain that their liberation is not due merely to manipulation of the blood. These free forms are undoubtedly present in the blood in the bird, scanty or numerous as the case may be. We are left somewhat uncertain, however, whether the process of liberation is an active or passive one on the part of the parasites, i. e. whether the Halteridia leave the red blood-cells of their own accord, by breaking out or away from them, or whether they are simply set free by the rupture of a used-up corpuscle, which has probably contained several parasites. Schaudinn maintained, of course, that the intra-cellular Halteridia regularly become active and trypaniform, and voluntarily leave the corpuscles. We should have seen this process in owls 19 and 23 if it occurs. On the whole, we are much more inclined to think the latter explanation we have suggested is the true one. In this connection it is important and interesting to note that there is markedly little or no hypertrophy and enlargement of the red blood-corpuscles by the Halteridia, even when there are several individuals in one host-cell; this contrasts strikingly with the great enlargement and stretching which the red cells may undergo when infected with Hæmogregarines or Hæmocystidium, for example. Hence it seems to us most likely that in an abundant infection, when there are three or more Halteridia in a corpuscle, their growth and increase in size renders it only a question of time before the corpuscle is ruptured, thus passively liberating the enclosed parasites. We should say it is very doubtful whether such free forms become again intra-cellular.

Mayer, in his account of the parasites of another owl, Syrnium aluco, also admits that he sought in vain for active trypaniform phases (trypanosome-forms) of the Halteridium.

Before leaving this question we should like to put forward

a suggestion, which is, however, nothing more than a suggestion. We think it is not at all impossible that there may be some intra-cellular phase of trypanosome, perhaps even occurring in a red blood-corpuscle, still to be definitely ascertained. As was discussed in the preceding memoir with reference to the recent work of Chagas (**2**) and Hartmann (**5**), it is reasonable to suppose that a schizogonous process of multiplication occurs in the life-history of these Avian trypanosomes, by which the small forms are developed from large, massive individuals. And it is quite possible that the immediate products of schizogony, which may be assumed to be small elements, pass for a time into the red cells before being liberated as small, active trypanosomes; such intra-cellular phases would most likely be found, we think, to be quite independent of the Halteridia, and might not possess pigment. In the scheme drawn up on p. 176, showing the relation between the different phases of the trypanosome, as found in the owl, we have indicated the position which this hypothetical intra-cellular form would occupy.

In this connection reference may perhaps be made to a remarkable passage in Zupitza's account of Avian trypanosomes (**19**). Zupitza states that in blood taken from a wounded "Haarvogel" ("Bülbül"), in which both trypanosomes and Halteridia were present, he observed two or three red blood-corpuscles which were undergoing a strange spontaneous movement, turning over now in one direction, now in another. Further wave-like swellings of the membrane or envelope of the corpuscle were noticed. According to Zupitza careful examination of these phenomena showed that they were due to a small, slender trypanosome inside the corpuscle, which coursed rapidly through the cytoplasm, around the nucleus, first in one sense and then in the opposite one. Apparently the trypanosome was trying to bore a way out through the envelope of the cell, but so long as Zupitza was able to watch it it was unsuccessful. We shall have to criticise Zupitza's paper in many respects, but we feel quite unable to offer any comment upon this wonderful description;

all we can do is to direct attention to it. Unfortunately, in the course of very numerous observations on birds infected with trypanosomes, neither of us has ever seen anything in the least corresponding.

Morphology of the Trypanosomes as seen in the Permanent Preparations.

All our permanent preparations are in the form of smears. Both the two chief methods of preparing films were employed, namely: (1) fixation by osmic acid vapour, followed by absolute alcohol, and then staining by Giemsa; and (2) wet fixation of smears made on cover-slips, either by sublimate-acetic mixture or by Schaudinn's fluid, and then staining by iron-hæmatoxylin. The details of our use of both these methods have already been described (see Minchin [**13**] and Minchin and Woodcock [**14**]), so they need not be repeated here. The drawings are all magnified 2000 times, so that a particular individual can be at once compared with any other.

The type or types of form presented by the parasites varied in different birds. In the early owls infected with trypanosomes (Nos. 8 and 13), three readily distinguishable types occurred, as has been indicated above in our account of the observations made on the living, active parasites. These three types may be distinguished as small, medium, and large respectively. While each type has sharply defined characteristics, and at the first glance might be regarded as representing a parasite quite distinct from both the others, nevertheless, as will be seen below, forms occur which are transitional in character from one type to the other, and there can be no doubt, we think, that all three are only different phases of one and the same trypanosome.

The small type of individual (figs. 1, 2, 14, 32–35) is spindle-like or fusiform in shape. The aflagellar extremity of the body is abrupt and conical, the kinetonucleus being situated close to the end. The undulating membrane is relatively quite well developed, and the free flagellum is fairly long

Using the standards of measurement previously adopted (loc. cit.), the total length of these forms, as they appear on smears made by the first (Romanowsky) method (figs. 1–3, 14), is from 26 to 28 μ, and the greatest width 3 to 4 μ, while the length of the free flagellum is usually about 8 to 9½ μ. The trophonucleus is situated about the middle of the body, or at most is only slightly nearer to the aflagellar end than to the other.

Comparing these forms as they are seen in wet films (i.e. films made by the second method), the length of the parasite appears uniformly somewhat less, averaging about 25 μ. There is no doubt that the body of the trypanosome is always rather contracted or shrunk by the wet method of preparation. This can be clearly seen to be the case by comparing figs. 32–35 of these small parasites on wet films with the figures of individuals of the same type on Giemsa smears. It will be noticed also that, in the former case, the flagellar border of the parasite frequently appears more crinkled and angular-looking than in the individual on "dry" smears; this being the result of a shortening of the body cytoplasm to a greater extent than the flagellar border (though the latter, and of course the free flagellum, is also somewhat contracted).

The next type of the parasite, the medium-sized form (figs. 7, 8, 20, and 21), is distinguished by its long, finely tapering aflagellar region, and also by the conspicuous undulating membrane, the folds of which are broad and high. The folds, it may be noted, frequently show the delicate endoplasmic intrusion, as described in the first memoir. The aflagellar end may be very narrow and attenuated (figs. 7 and 20). The free flagellum is relatively long. The entire length of these forms, in the above typical instances, is from 44–47 μ, the greatest width varies from 5 to 5½ μ, the length of the free flagellum is 11 to 13 μ, while the aflagellar prolongation of the body, measured from the kinetonucleus, is usually 5 to 6½ μ, but in the individual of fig. 7 it is as much as 9 μ. In this type the trophonucleus is always in the aflagellar half of the the body, i.e. it is nearer to the kinetonucleus than to the

point where the cytoplasm ends and the flagellum becomes free.

The cytoplasm of both the types of form just described is coloured either pale blue or a faint lilac in Giemsa smears. Usually it appears fairly homogeneous in character (figs. 20, 21), but now and then it contains granules, more or fewer, which stain dark red (fig. 8).

The third distinct variety of form shown by the trypanosome is very large, and also differs markedly from the other two types in the appearance of the cytoplasm (figs. 9–11, 22, 24). This is very dense, and in Giemsa-stained smears is coloured a deep and intense blue, which may be slightly tinged with purple or lilac. The aflagellar region of the body is prolonged for some distance beyond the kinetonucleus. It usually tapers to a fine extremity, but it is never so narrow and attenuated as in the medium-sized parasites, because in these large forms the body is much broader at the level of the kinetonucleus than in the latter. The undulating membrane may be very prominent, especially towards the flagellar end of the body. The dimensions of some of the largest of these forms (figs. 10, 22 and 24) are as follows: Total length 54–60 μ, greatest width $5\frac{1}{4}$–6 μ, length of aflagellar prolongation 12–15 μ, and length of free flagellum $8\frac{1}{2}$ μ, the last named being comparatively short. A rather shorter but broader individual (fig. 11) measures 50 μ in length, $6\frac{1}{2}$ μ in width, while the aflagellar part is 9 μ long, and the flagellum again $8\frac{1}{2}$ μ.

In wet films, stained by iron-hæmatoxylin, this type is readily distinguishable (figs. 43 and 44), both by its general shape, which agrees quite well with appearance of similar parasites on the other smears (allowing for a uniform shrinkage in size), and also by the staining reaction of the cytoplasm, which is stained dark grey, a much deeper tint than in the case of the small or medium-sized individuals, whose cytoplasm is quite pale or else only slightly stained. The largest examples of this type, as seen in wet films, measure 44 μ in length, $4\frac{1}{2}$ μ in breadth, the aflagellar region is 7 μ long, and

the free flagellum 7–10 μ; it may be, of course, that these particular examples are not really quite as large as that, for instance, of fig. 10, making allowance for some contraction.

All the individuals of this large type which we have found in wet films have the same general shape and appearance—that of a long, rather thick spindle with finely tapering extremities; and this form agrees closely with the appearance of these individuals as they were observed alive. Hence we feel sure that on Giemsa-stained smears, those individuals which closely resemble the above-described parasites on wet films can be correctly regarded as having retained the typical and normal appearance; and it is from such standard examples that we have taken the measurements given above. This is an important point to note, because these large parasites are difficult to obtain well fixed and stained on a Giemsa smear. Not uncommonly they are found of the weirdest shape and appearance; we have not the least doubt that such individuals have been deformed and distorted in making the preparation. Such parasites are generally very much flattened out, while the aflagellar end is blunt and broad and has quite lost its true shape; sometimes the whole trypanosome may appear nearly rectangular. It is quite useless to figure such individuals; Zupitza (**19**) has given (fig. 49, pl. 5), an excellent illustration of how far removed from its true shape one of these large forms (which he regards as "T. ziemanni") may appear on a dry, Giemsa-stained smear. Unfortunately, from his description Zupitza apparently quite fails to realise that the individual he figures is hopelessly flattened out and distorted.

The question of the flattening-out of these large forms, which is, of course, liable to occur on a "dry" film, has an important bearing, we think, on another point. In the memoir on the trypanosome of the chaffinch and redpoll, the corresponding large blue forms there described showed in most cases a characteristic structural peculiarity of the cytoplasm, namely its tendency to show an arrangement into longitudinal bands, dark and light alternating, the former

apparently composed of coarser, more closely packed granules. It was stated then that these bands were not to be regarded as actually representing myonemes. Now, in the case of the large blue trypanosomes from the little owl, most of the well-fixed individuals, i.e. those most closely approximating in form (shape) to the corresponding type on wet films, show scarcely any indications of such a structural differentiation of the cytoplasm. Only in one or two individuals, which are relatively rather wide, can traces of the bands be made out (cf. fig. 11). And none of these forms on wet films shows any signs of this peculiarity, the cytoplasm being practically homogeneous; this fact is, we think, most instructive. We have come to the conclusion that this appearance is, to a large extent, artificial, and chiefly the result of a certain flattening-out on the "dry" Giemsa-stained smears. The narrower and more compact the general cytoplasm of the parasite, the less conspicuous is this band-like arrangement.

It may be asked, Why was this condition found very frequently in the large forms of T. fringillinarum, which were figured as normal? It must be recalled in explanation that the preparations on which the account of these forms in the latter trypanosome was based differed in two respects from those we have of the parasites from the little owl. In the first place, no wet films of T. fringillinarum were obtained. Secondly, the smears were made from the peripheral blood, and were, of course, evenly spread out and thin. On the other hand, in the case of the trypanosome with which we are now concerned, all our preparations showing these large individuals are made from the bone-marrow, and the smears are not nearly so thinly spread out. In fact, the best fixed examples of these large forms are those which occur in the neighbourhood of clumps and masses of cells.

It is fairly certain, therefore, that in the blood-smears containing T. fringillinarum, these large blue parasites were, while really not distorted, nevertheless sufficiently flattened-out to produce this effect of bands in the cytoplasm. At the time, however, there was no reason why one should have con-

cluded this to be the case. If the figures given in the preceding memoir of the large forms of T. fringillinarum are compared with figs. 10 and 24 accompanying of the corresponding type of the parasite of the little owl, it will be noted that the general cytoplasm in the former appears distinctly wider in proportion to the length of the body than in the latter trypanosome, although this is really a larger (longer) species. It may be mentioned that in one of the figures (fig. 48, pl. 5) which Zupitza (loc. cit.) gives of the large trypanosome identified by him with "T. ziemanni" from Eurystomus afer, showing an individual that is manifestly flattened out, the cytoplasm also shows distinct bands.

As regards the significance of this appearance we think it is probable that the clearer, lighter longitudinal zones, which are usually the narrower, may correspond to the position of the myonemes, though they do not, of course, actually represent them. The myonemes themselves are most probably fine but definite lines; apparently they are not easily demonstrable in Giemsa-stained preparations. One of us (**13**) was fortunate to secure a preparation of T. percæ, made by the wet method, which showed the myonemes well, but even in the case of the large T. raiæ, in iron-hæmatoxylin-stained films, we were unsuccessful (**14**) in seeing them; also in none of our wet preparations of the larger forms of the trypansome of the owl have we been able to make them out.

Of the three types or phases of the trypanosome above described, which occur together in the bone-marrow, and there alone, the small forms are the most numerous, the big blue individuals are distinctly less frequent, while the slender, medium-sized type, in its most fully developed condition with the long, narrow aflagellar prolongation is least common, and occurs somewhat scantily.

As already indicated, these three phases can be definitely connected with one another by means of transitional forms which occur. Thus the medium-sized, slender parasites arise from the small ones by growth and extension of the

body, principally in the direction of length ; at the same time the aflagellar prolongation becomes conspicuously developed.

A regular series of intermediate stages is seen, for example, in figs. 4, 18, 17, 6 and 19. The dimensions of these individuals are as follows: Fig. 4, (*a*) total length 35 μ, (*b*) width $2\frac{1}{2}$ μ, (*c*) free flagellum 11 μ, (*d*) aflagellar part 4 μ; fig. 18, (*a*) 40 μ, (*b*) $2\frac{3}{4}$ μ, (*c*) 16 μ, (*d*) 3 μ; fig. 6, (*a*) 39 μ, (*b*) 3 μ, (*c*) $9\frac{1}{2}$ μ, (*d*) 5 μ; fig. 17, (*a*) 42 μ, (*b*) $3\frac{1}{2}$ μ, (*c*) 12 μ, (*d*) 4 μ; fig. 19, (*a*) 43 μ, (*b*) 4 μ, (*c*) 11 μ, (*d*) $5\frac{1}{2}$ μ. It will be noticed that the length of the free flagellum may vary not inconsiderably and apparently indiscriminately in these forms. This point was referred to in describing the cultural forms of T. fringillinarum; and we think it is most probable that the explanation given in that case holds here also, namely, that the different length of the flagellum in what are otherwise similar individuals is chiefly the result of the unequal splitting of this organella in dividing parasites.

In T. fringillinarum, it may be remembered, certain of the small forms occurring in the bone-marrow, which were broader and more stumpy than the others, were found showing unmistakably commencing division (cf. fig. 54 of the earlier memoir). Hence, as regards the corresponding small forms from the little owl, it is most likely that they also divide by binary fission, although apparently most infrequently. We have not been able to secure as marked indications of the process as were obtained in T. fringillinarum, but we have found individuals which are broader or stouter than the rest, some of which showed the kinetonucleus double (fig. 16). Just as in the case of the chaffinch-parasite, these small trypanosomes are the only type of form in which we have been able to find even a hint of binary division.

Next, with regard to the large "blue" parasites, these also can be undoubtedly linked up to medium-sized, slender forms by intermediate stages, such as those shown in figs. 9 and 23. The measurements of these two individuals are as follows: Fig. 9, length 43 μ, width $3\frac{1}{2}$ μ, free flagellum 7 μ, aflagellar prolongation 8 μ; and fig. 23, length 39 μ (at

least),[1] breadth 4½ μ, free flagellum 7 μ (at least), and aflagellar portion 6 μ. It will be seen that from medium-sized forms, such as those of figs. 6, 17 and 5 (which have not attained the extremely elongated appearance presented by this type, when fully developed), it is a very slight step to the "blue" forms of figs. 9 and 23.

The latter type arises, we should say, principally by an increase in bulk or density of the general cytoplasm of the body, which results in a distinct alteration in its staining reaction (chiefly by Giemsa). It is important to note that this in no way involves a contraction in length, i.e. there is no shortening of the body to compensate for increased stoutness. On the contrary, in the larger individuals the whole body, including the aflagellar portion, is found to have increased in length as well as in width or bulk. We shall have to refer again to this point when subsequently criticising Zupitza's paper.

In the later owls (Nos. 19 and 23) the trypanosomes are in a quite different phase—that is to say, the parasites are in what we regard as the summer condition. We have not observed any individuals either of the slender, medium-sized variety, or of the large blue type; both these forms appear to be entirely absent. Neither do most of the parasites present in these later owls quite correspond to the small forms occurring in the earlier birds, though in all probability they are developed from the latter type.

The summer forms of trypanosome (figs. 12, 13, 29–31, 40–42) are all fusiform and stumpy in character, with the kinetonucleus situated near the abruptly pointed aflagellar end; nearly all the individuals observed, however, are distinctly larger than the small forms above described. Moreover, the body, instead of being a fairly slender spindle, is, in most cases, comparatively thick or stout in proportion to its length. This character may appear to be very marked, both

[1] The total length of the parasite, as also the length of the free flagellum, is probably a few μ longer, since the end of the flagellum pasess over the nucleus of a blood-cell, where it cannot be traced.

among the smaller and the larger individuals met with, so much so that many of these parasites show in stained preparations what has been described as a leaf-like appearance or shape (figs. 12, 30, and 31). This is seen chiefly in smears made from the peripheral blood, and is only exceptionally found in the case of individuals on smears made from the bone-marrow. Here also we have come to the conclusion that this wide, leaf-like appearance is largely or almost entirely due to the (artificial) flattening-out of thick, fusiform parasites on thinly spread smears. This view is borne out by a comparison of this type of parasite as it occurs in films prepared by the wet method and stained by iron-hæmatoxylin. All the parasites observed have the form of a stout spindle (figs. 40–42); but no leaf-like individuals have been seen on wet films. Moreover, in the leaf-like forms the trophonucleus appears more or less transversely elongated (figs. 12, 29, 30), which is never the case in any other form of the parasite; compare Minchin (**13**, p. 17) on similar forms of Trypanosoma percæ. Hence, in arriving at a correct estimate of the proportions of the summer type of the parasite, such flattened-out individuals are best left out of consideration, since their breadth probably appears considerably greater than is actually the case.

Fig. 13 shows the typical appearance of a trypanosome of this stout, fusiform type, this being a fairly large individual. Its length is 32 μ, greatest breadth or thickness (including the undulating membrane) $4\frac{3}{4}$ μ, and the length of the free flagellum 9 μ. Rather smaller parasites are seen in figs. 25 and 26, the former being 30 μ long, $4\frac{1}{4}$ μ broad, and the free flagellum 7 μ, while the latter is 34 μ long, accounted for by the much longer flagellum of 12 μ, and $4\frac{1}{2}$ μ broad. Of the flattened, more leaf-like individuals those of figs. 31 and 30 correspond in reality closely, we have no doubt, to the above-mentioned parasites. The former is 32 μ long, the free flagellum being $6\frac{1}{2}$ μ, and its width appears to be $6\frac{1}{2}$ μ; the latter is $29\frac{1}{2}$ μ long, the flagellum being 8 μ, while the width is apparently as much as $7\frac{1}{2}$ μ. This latter parasite

shows, it will be noticed, the curious chain of granules running parallel to the flagellar border, which was frequently observed in the case of the corresponding forms of T. fringillinarum. The smallest trypanosome of this type which has been observed (fig. 29) also happens unfortunately to be on a thin blood-smear,[1] and is leaf-like. It is only $23\frac{1}{2}\,\mu$ long, the free flagellum being $6\frac{1}{2}\,\mu$, and it appears to be as much as $6\frac{1}{2}\,\mu$ wide! Individuals as small as this are very exceptional; the majority appear to be of much the same size, having an average length of about $30\,\mu$, and not differing greatly from the dimensions given above.

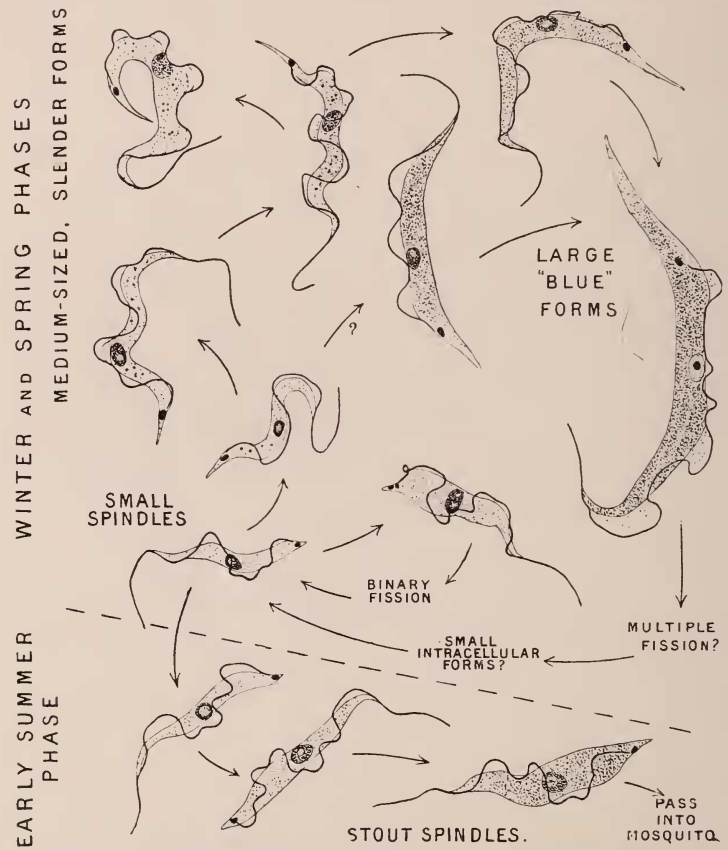
Comparing now this type of form as it occurs on wet films, we find, as already indicated, that all the parasites are fairly uniform in size and appearance. They show well the typical thick, fusiform shape of the body. They also exemplify another important point, namely, the considerable shrinkage in size which is undergone by the trypanosomes in the course of preparing the wet films. The total length averages about $23\,\mu$, the individual of fig. 42, for instance, being $25\,\mu$ long by $4\frac{1}{4}\,\mu$ broad, while that of fig. 41 is $20\frac{1}{2}\,\mu$ by $3\frac{3}{4}\,\mu$. We have no hesitation in comparing these forms with the average-sized ones of the dry series—say, for instance, figs. 13 and 30 respectively. It might be thought, perhaps that the parasites of figs. 40–42 corresponded rather to the very small individual of fig. 29. We certainly do not think so. We have only found such a small form in that one case, the average size being considerably larger (longer), and the wet films were made at the same time as the Romanowsky ones. Further, as was shown above, there is relatively quite as great shrinkage in what are undoubtedly medium-sized slender forms and large "blue" ones (i. e. types found in the earlier birds) on wet films.

A feature in which the stout spindles differ markedly from the slender small forms is the much larger size of the tropho-

[1] On one or two thick blood-smears which were made, nearly all the parasites have the more typical form, only a few individuals in the thinner parts tending to be flattened out.

nucleus, particularly of the karyosome. This is well brought out by iron-hæmatoxylin-stained films (figs. 40–42), but it is also seen in those Giemsa-stained individuals in which the

Text-fig. 1.



Scheme showing the different types of form of Trypanosoma noctual and their connection with one another.

karyosome appears as a clear area in the nucleus (figs. 12 and 13).

General Considerations.

The principal conclusion at which we have arrived in this paper is that all the different forms of trypanosome in the

blood of Athene noctua belong to one and the same species, for which we use the name Trypanosoma noctuæ, Schaudinn, in spite of the differences between Schaudinn's figures and ours. Above is a scheme showing the connection between the different types of individual here described.

The stout spindles, which are the only forms we have found in the summer condition of the trypanosome, and which occur, it will be remembered, also in the general circulation, are undoubtedly a transmissive phase of the parasite; on passing into a mosquito (Culex) they give rise, as will be shown in a subsequent memoir, to the developmental forms characteristic of the Insectan host. It is interesting and important to note that an exactly similar state of affairs was found to be present in the trypanosome of the chaffinch in its summer phase (cf. Case B in the former memoir).

As indicated in the scheme, these stout spindles arise, we feel practically certain, from individuals of the small fusiform type, such as occurred in the earlier birds, their development being along rather a different line from that leading to the medium-sized and large "blue" types (the early spring phases). Individuals such as that shown in fig. 27 are clearly transitional between the small type and the fully-developed stout spindle. While the parasite increases in length to a certain, but not very great extent, the principal direction in which growth or development takes place is a pronounced increase in thickness or stoutness of the body (cf. figs. 32 and 33 and 40 and 42 respectively, from wet films).

Zupitza (loc. cit.) has, it seems to us, a quite erroneous idea about the origin of these stout forms (and, what are the same thing, the leaf-like forms). He considers them as being later (older) stages of long, slender (so-called "spirochætiform") parasites. They are regarded as being developed by a process of thickening or swelling of the body-protoplasm, chiefly in the middle; concurrently the kinetonuclens is pushed further towards the aflagellar end, and the delicate

aflagellar prolongation is gradually lost, this region of the parasite ultimately taking on the short, abrupt appearance characteristic of the stout spindles. We certainly have never found any indication of such a process, either in the trypanosome of the little owl or in T. fringillinarum from the chaffinch. In those types of form which develop a marked aflagellar prolongation, this becomes distinctly more prominent with the growth of the individual, corresponding with the increase in size of the rest of the body. This is the case both with the medium-sized, tapering forms (which were termed "definitive" forms in the first memoir [18]), and the massive "blue" forms, as is clearly shown by a comparison of the series of figures, both of the parasite of the owl and that of the chaffinch. On the other hand, in the case of both the distinct trypanosomes with which these studies have been concerned, the stout spindles can be readily connected by transitional forms with the small fusiform type (cf. particularly the figs. 5, 6, 42–44 of T. fringillinarum, which show a complete series of intermediate stages). Another point to be noted is that the larger individuals of the slender, tapering type have considerably more bulk than the smaller individuals of the stout spindle type, and could by no possibility become the latter. Zupitza's account gives, indeed, a somewhat confused idea of the different phases of Avian trypanosomes and their relation to one another. This is partly due to the fact that he compares trypanosomes from quite different hosts as if they were all phases in the life-cycle of one and the same parasite, without having regard to possible differences in size of the various forms, instead of studying the same form in the same host through different seasons of the year.

We have not been able to observe the condition or phase in which the trypanosomes occur in the owl in the early autumn, not having had any infected birds at this season. Hence, we do not know what becomes of the stout spindles, which do not pass into the Insectan host. As was discussed in the case of T. fringillinarum, we think it quite likely such forms

may develop further into large "blue" forms; these were found in the general circulation in the autumn in the case of T. fringillinarum.

The chief, and, indeed, about the only point of difference between the trypanosome of the little owl and T. fringillinarum, so far as we have observed the two parasites, is that in the latter species the massive forms were not found in association with the small forms and the medium-sized ("definitive") individuals in the early spring, as in the former parasite. It is quite possible, however, that these forms could be found at that period in T. fringillinarum also.[1] Hence we do not now consider that there is much to be gained by continuing to distinguish the medium-sized slender individuals as "definitive" forms. In the case of the owl-trypanosome this phase does not appear to be so prominent or persistent as was found to be the case in T. fringillinarum. It was mentioned in describing that species that the "ordinary" forms, as they were regarded, might pass later into the massive "blue" type. As we have shown above in the present case there is undoubtedly a transition from medium-sized slender forms to the large blue type; though we are uncertain whether the former phase in its most fully developed condition (cf. figs. 7 and 8) undergoes this further development.

From the marked correspondence as regards the different types of form which we have found in the case of two species from widely different hosts we venture to think that the scheme drawn up on p. 176 may be regarded as applicable in the main to other Avian trypanosomes, and may perhaps be taken as indicating typical phases in the life-cycle occurring in the bird which are common to most species. Reading Zupitza's paper in this light we find that several points of agreement with our results are shown by the parasites with which he worked. The same types of individual are described

[1] Since this paper was sent to press, one of us (H. M. W.) has found these large forms in a chaffinch infected with T. fringillinarum early in May.

or figured under one name or another, and from one or more birds. The true stout spindles, it may be noted, which type is distinguished by Zupitza as "T. avium minus" occurred in most instances alone, just as we found to be the case.[1] Massive blue forms were also met with (termed in one case "T. avium majus" and in another T. ziemanni).

From the descriptions which have been given of the trypanosome of the little owl and of T. fringillinarum in this and the preceding memoir, it will be clearly seen, we think, that these various names, such as T. avium minus, T. avium majus, etc., can be regarded only as general designations for different types or phases which occur in the life-cycle of, at any rate, many species of Avian trypanosome. They do not represent distinct and independent forms or varieties. A further very important point brought out is that this applies also to the type which has been hitherto distinguished as T. ziemanni. "T. ziemanni" is really only the large "blue" phase of T. noctuæ, the trypanosome parasitic in Athene noctua. This is equally true, we have no doubt, for the species parasitic in Syrnium aluco, whether that is to be considered also as T. noctuæ, or as being a distinct species.[2] Mayer (**12**), in his recent paper on the parasites of this latter owl, which will be more fully dealt with in a subsequent memoir, figures trypanosomes which belong both to the stout spindle type and to the large massive forms, the latter being regarded as "Leucocytozoon-forms," i. e. as equivalent to Schaudinn's T. ziemanni.

As we stated in the earlier part of this paper we have seen nothing in the case of the parasites of the little owl to lend any support to the view that these large trypanosomes are actually connected with the Leucocytozoon ziemanni. In the first place the latter parasite, in its large form, always

[1] Unfortunately we cannot gather from Zupitza's account whether his birds were all examined at the same season of the year, or at different periods.

[2] If it is a distinct species, to it belongs the name T. avium, as emended by Laveran (**6**).

occurs in the gametocyte phase, i. e. in the character of male or female individuals which give rise only to the sexual elements. Moreover, we have never found any corresponding sexual difference among the massive trypanosomes which might indicate that they were of male or female character. They never show the marked differences in staining reaction presented by the Leucocytozoon.

We may repeat, therefore, that "T. ziemanni" is to be regarded only as a phase or type of form of T. noctuæ, just as similar large forms occur in T. fringillinarum; and again, for instance, in the trypanosome parasitic in Eurystomus afer (allied to the roller-bird), where they are also regarded by Zupitza (loc. cit.) as "Leucocytozoon-forms" and designated "T. ziemanni"![1]

In view of the general agreement which we have found between the different types of T. noctuæ and those of T. fringillinarum, why, it may be asked, do we not regard both these parasites, for instance, as belonging to the same species? We feel practically certain that they are quite distinct forms for the following reasons: First, on the ground of their occurrence in very different birds, the hosts being respectively little owls (and perhaps also other owls), and chaffinches (and also redpolls and perhaps other Fringillinæ). Secondly, we have on several occasions inoculated a little owl, free from any blood-parasites, with cultural forms of T. fringillinarum, but have been unsuccessful in obtaining any development of the trypanosomes in the bird. Lastly, on morphological grounds, the various types of form of T. noctuæ appear to attain a slightly but distinctly larger size than do the corresponding types of T. fringillinarum so far as we can judge from our own observations. This is seen from the following table comparing the two sets of forms:

[1] In any case, it is most unlikely that this parasite, occurring in a quite different bird, would be the same species as Schaudinn's "T. zeimanni."

Type of form.	T. fringillinarum.	T. noctuæ.
A. Small forms (slender spindles), smallest individuals observed	Total length, 25 μ. Greatest breadth, 3$\frac{1}{4}$ μ	Total length, 26$\frac{1}{2}$ μ. Greatest breadth, 3$\frac{1}{2}$ μ.
B. Medium-sized, slender forms (so-called "definitive" forms), average size of fully developed individuals	Total length, 41–45 μ Greatest width, 4$\frac{1}{2}$–5 μ	Total length, 44–47$\frac{1}{2}$ μ. Greatest width, 5–5$\frac{1}{2}$ μ.
C. Large, massive "blue" forms, average size of largest individuals	Total length, 45–48 μ Greatest breadth, 5$\frac{3}{4}$–6$\frac{1}{2}$ μ (probably slightly flattened out—see text)	Total length, 54–60 μ. Greatest breadth, 5$\frac{1}{4}$-6 μ.

The Lister Institute,
April, 1911.

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EXPLANATION OF PLATES 20 AND 21,

Illustrating Observations by Messrs. E. A Minchin, M.A., F.R.S., and H. M. Woodcock, D.Sc., on "The Trypanosome of the Little Owl (Athene noctua), with Remarks on the other Protozoan Blood-parasites Occurring in this Bird."

[All the figures relate to Trypanosoma noctuæ, and are magnified 2000 times linear. We are indebted to Miss Rhodes for kindly drawing and colouring most of the figures on Pl. 20, and for drawing two or three of those on Pl. 21.]

PLATE 20.

Figs. 1–11 are from owl 8; figs. 12 and 13 from owl 23.

(All the figures are from preparations stained by Giemsa.)

Figs. 1–3.—Small forms (small spindles).

Fig. 4.—Transitional form from small fusiform type to slender, medium-sized type.

Figs. 5 and 6.—Young individuals of the slender, medium-sized type (not fully developed).

Figs. 7 and 8.—Fully developed individuals of the medium-sized form; the individual of fig. 7 is probably a little flattened.

Fig. 9.—Small individual of the massive "blue" type (c f. with figs. 5 and 6).

Figs. 10 and 11.—Large individuals of the massive "blue" type; the latter shows indications of the longitudinal bands in the cytoplasm.

Figs. 12 and 13.—Stout spindles (early summer phase); the former is a typical, full-sized individual; the latter is flattened out (so-called leaf form).

PLATE 21.

Figs. 14–17, 19 and 20, 22–24, 32–36, 38, 39 and 44 are from owl 8; figs. 18, 21, 37 and 43 from owl 13; figs. 25 and 26 from owl 19; and figs. 27–31, 40–42 from owl 23.

(Figs. 14–31 are from preparations stained by Giemsa.)

Figs. 14 and 15.—Small spindles.

Fig. 16.—Small spindle, just commencing division; there are two kinetonuclei, and apparently the karyosome in the trophonucleus has also divided.

Figs. 17–19.—Intermediate stages in the growth of the medium-sized slender type.

Figs. 20 and 21.—Fully developed medium-sized forms.

Figs. 22 and 24.—Massive "blue" forms.

Fig. 23.—Small individual of the same type.

Figs. 25, 26, and 28.—Stout spindles.

Fig. 27.—Transitional form from small fusiform type to stout spindle.

Figs. 29–31.—Stout spindles, more or less flattened-out (leaf-like); the individual of fig. 29 is the shortest of this kind found.

(Figs. 32–44 are from preparations stained by iron-hæmatoxylin.)

Figs. 32–35.—Small spindles.

Figs. 36–39.—Medium-sized slender forms.

Figs. 40–42.—Stout spindles.

Figs. 43 and 44.—Massive "blue" forms.